

CLAIMS

1. A fluorescent protein derived from *favia favius* having the following properties:
 - (1) an excitation maximum wavelength is 507 nm;
 - (2) a fluorescence maximum wavelength is 517 nm;
 - (3) a molar absorption coefficient at 482 nm is 80,000;
 - (4) a quantum yield is 0.68; and
 - (5) pH sensitivity of the fluorescence maximum is stable at pH 5 to pH 11.
2. A fluorescent protein having any of the following amino acid sequences:
 - (a) an amino acid sequence shown in SEQ ID NO: 1; or
 - (b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and having fluorescent properties.
3. A fluorescent protein having an amino acid sequence derived from any of the following amino acid sequences by substitution from asparagine to histidine at 62th amino acid residue:
 - (a) an amino acid sequence shown in SEQ ID NO: 1; or
 - (b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and having fluorescent properties.
4. A fluorescent protein having an amino acid sequence derived from any of the following amino acid sequences by substitution from methionine to valine at 40th amino acid residue, substitution from aspartic acid to histidine at 62th amino acid residue, and substitution from isoleucine to methionine at 198th amino acid residue.
 - (a) an amino acid sequence shown in SEQ ID NO: 1; or
 - (b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO:

1, and having fluorescent properties.

5. A fluorescent protein having an amino acid sequence derived from any of the following amino acid sequences by substitution from methionine to isoleucine at 10th amino acid residue, substitution from leucine to valine at 12th amino acid residue, substitution from methionine to valine at 40th amino acid residue, substitution from valine to alanine at 60th amino acid residue, substitution from aspartic acid to histidine at 62th amino acid residue, substitution from tyrosine to asparagine at 119th amino acid residue, substitution from proline to serine at 144th amino acid residue, substitution from arginine to leucine at 197th amino acid residue, and substitution from isoleucine to methionine at 198th amino acid residue:

(a) an amino acid sequence shown in SEQ ID NO: 1; or

(b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and having fluorescent properties.

6. A fluorescent protein having an amino acid sequence derived from any of the following amino acid sequences by substitution from methionine to isoleucine at 10th amino acid residue, substitution from methionine to valine at 40th amino acid residue, substitution from valine to alanine at 60th amino acid residue, substitution from aspartic acid to histidine at 62th amino acid residue, substitution from lysine to glutamic acid at 70th amino acid residue, substitution from tyrosine to asparagine at 119th amino acid residue, substitution from arginine to glutamine at 197th amino acid residue, and substitution from isoleucine to methionine at 198th amino acid residue:

(a) an amino acid sequence shown in SEQ ID NO: 1; or

(b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and having fluorescent properties.

7. A fluorescent protein having an amino acid sequence derived from any of the

following amino acid sequences by substitution from valine to alanine at 60th amino acid residue, substitution from aspartic acid to glycine at 62th amino acid residue, substitution from tyrosine to histidine at 63th amino acid residue, substitution from histidine to leucine at 196th amino acid residue, and substitution from isoleucine to threonine at 198th amino acid residue:

- (a) an amino acid sequence shown in SEQ ID NO: 1; or
- (b) an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and having fluorescent properties.

8. A DNA encoding the protein of the present invention.

9. A DNA of any of the following:

- (a) DNA encoding the amino acid sequence shown in SEQ ID NO: 1; or
- (b) DNA encoding an amino acid sequence comprising a deletion, substitution and/or addition of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and encoding a fluorescent protein.

10. A DNA having any of the following nucleotide sequences:

- (a) a nucleotide sequence shown in SEQ ID NO: 2; or
- (b) a nucleotide sequence comprising a deletion, substitution and/or addition of one or several nucleotides with respect of the nucleotide sequence shown in SEQ ID NO: 2, and encoding a fluorescent protein.

11. A DNA comprising any of the following nucleotide sequences:

- (a) a nucleotide sequence shown in SEQ ID NO: 13, 15, 17, 19, or 21; or
- (b) a nucleotide sequence comprising a deletion, substitution and/or addition of one or several nucleotides with respect of the nucleotide sequence shown in SEQ ID NO: 13, 15, 17, 19, or 21, and encoding a fluorescent protein.

12. A recombinant vector having the DNA of any of claims 8 to 11.

13. A transformant having the DNA of any of claims 8 to 11 or the recombinant vector of claim 12.

14. A fusion fluorescent protein consisting of the fluorescent proteins of any of claims 1 to 7 and another protein.

15. The fusion fluorescent protein of claim 14 wherein said another protein is one that localizes in the cell.

16. The fusion fluorescent protein of claim 14 or 15 wherein said another protein is one specific to an intracellular organella.

17. A method for analyzing the localization or dynamics of a protein in cells, characterized in that the fusion protein of any of claims 14 to 16 is allowed to be expressed in cells.

18. A fluorescent reagent kit which comprises any of the fluorescent protein of any of claims 1 to 7, the DNA of any of claims 8 to 11, the recombinant vector of claim 12, the transformant of claim 13 or the fusion protein of any of claims 14 to 16.

19. A method for producing a fluorescent protein capable of green-to-red photoconversion in response to irradiation with light, which comprises introducing substitution from an amino acid corresponding to 62th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to histidine into a fluorescent protein incapable of green-to-red photoconversion in response to irradiation with light.

20. A method for producing a fluorescent protein capable of green-to-red photoconversion in response to irradiation with light, which comprises introducing at least one of the following amino acid substitutions into a fluorescent protein incapable of green-to-red photoconversion in response to irradiation with light;

(1) substitution from an amino acid corresponding to 62th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to histidine;

(2) substitution from an amino acid corresponding to 10th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to isoleucine;

(3) substitution from an amino acid corresponding to 12th amino acid residue of

the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to valine;

(4) substitution from an amino acid corresponding to 40th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to valine;

(5) substitution from an amino acid corresponding to 60th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to alanine;

(6) substitution from an amino acid corresponding to 70th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to glutamic acid;

(7) substitution from an amino acid corresponding to 119th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to asparagine;

(8) substitution from an amino acid corresponding to 144th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to serine;

(9) substitution from an amino acid corresponding to 197th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to leucine or glutamine; or

(10) substitution from an amino acid corresponding to 198th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to methionine;

21. A method for producing a fluorescent protein with an increased rate of green-to-red photoconversion and enhanced fluorescence intensity, which comprises introducing at least one of the following amino acid substitutions in a fluorescent protein:

(1) substitution from an amino acid corresponding to 62th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to histidine;

(2) substitution from an amino acid corresponding to 54th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to phenylalanine;

(3) substitution from an amino acid corresponding to 69th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to valine;

(4) substitution from an amino acid corresponding to 87th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to tyrosine;

(5) substitution from an amino acid corresponding to 93th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to methionine;

(6) substitution from an amino acid corresponding to 109th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to methionine;

(7) substitution from an amino acid corresponding to 121th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to isoleucine;

(8) substitution from an amino acid corresponding to 140th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to valine;
or

(9) substitution from an amino acid corresponding to 160th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to valine;

22. A method for producing a fluorescent protein capable of purple-to-blue photoconversion in response to irradiation with light, which comprises introducing at least one of the following amino acid substitutions in a fluorescent protein incapable of purple-to-blue photoconversion in response to irradiation with light:

(1) substitution from an amino acid corresponding to 60th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to alanine;

(2) substitution from an amino acid corresponding to 62th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to glycine;

(3) substitution from an amino acid corresponding to 63th amino acid residue of

the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to histidine;

(4) substitution from an amino acid corresponding to 196th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to leucine;
or

(5) substitution from an amino acid corresponding to 198th amino acid residue of the fluorescent protein having the amino acid sequence shown in SEQ ID NO: 1 to threonine;